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NOVEL TOXINS AND BIOREGULATORS:

THE EMERGING SCIENTIFIC AND TECHNOLOGICAL ISSUES RELATING TO VERIFICATION AND THE BIOLOGICAL AND TOXIN WEAPONS CONVENTION

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FOREWORD

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This was a firm government commitment, made well before the 1972 Biological and Toxin Weapons Convention (BTWC) had entered an advanced stage of negotiation. Canadian policy is no less clear today: in 1972, Canada ratified the BTWC; and, at the 1989 Paris Conference on the Geneva Protocol, the Canadian position of 1970/71 was reiterated by the (then) Secretary of State for External Affairs, the Right Honourable Joe Clark. The third review conference of the Biological and Toxin Weapons Convention provides a welcome opportunity to reinforce everyone's understanding of the depth of the Canadian commitment to the abolition of such terrible weapons, and to express Canada's continuing willingness to cooperate with others in the global undertaking represented by the Convention.

FOREWORD

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PREFACE

In preparing for the Third Review Conference of the Biological and Toxin Weapons Convention, much has been said about the need to strengthen the Convention. Part of this discussion involves proposals to amend the Convention to include verification provisions, quite apart from other efforts, past and future, to consider the addition of voluntary confidence-building measures.

Before one can talk about verification or confidence-building in a substantive way, it is important to have a clear understanding of the range of materials and activities that might need to be addressed by such schemes. In the case of the Biological and Toxin Weapons Convention, there have been significant improvements in the nature and number of technologies and facilities used in the production of materials relevant to the Convention. These advances, then, are also relevant to the nature and scope of any potential verification regime or confidence-building measures. This paper focuses on these technological changes, suggesting some of the considerations that will need to be taken into account should it be decided to attempt to improve upon the existing Convention.

In attempting to provide some historical background on the way toxins were regarded in the past, many different viewpoints had to be compressed into a few paragraphs. The compromises may not find universal acceptance but, since such material is not the focus of this document, these historical references should be regarded only as illustrative of the discussion that has taken place in the open literature.

The Canadian Government wishes to acknowledge the work performed under contract by Brac Scientific Consulting, in collaboration with the Verification Research Unit of External Affairs and International Trade Canada. All of the material discussed in this publication is derived from readily available open sources.

Apart from the preceding Foreword, the views expressed herein do not necessarily reflect the views of the Canadian Government as they exist today or as they will eventually materialize from the international consultative process. However, it was considered that this document would be of interest to other States Parties to the Biological and Toxin Weapons Convention, and so it was agreed to give it wider distribution in order to promote discussion.

GLOSSARY

Amino acid. An organic acid containing amino and carboxyl chemical groups: amino acids are the building blocks of peptides, polypeptides and proteins.

Peptide. A molecule formed by the linking together of two or more amino acids: peptides may have up to fifty amino acids.

Polypeptide. A string of fifty or more amino acids linked together.

Protein. Two or more polypeptide chains linked together for a specific function.

Toxin. Naturally occurring chemical compounds isolated from animals, plants or microorganisms, or their synthetic analogues that have toxic effects on humans or animals. Most of the toxins discussed in this paper are peptides.

Bioregulator. Chemical compounds produced by cells in one part of an organism that have profound regulatory effects on biological processes within the organism; most recently discovered bioregulators are small peptides.

Analogue (also **Analog**): A modified form of the original molecule of the toxin or bioregulator, made by chemical synthesis, genetic engineering, or any other means. Analogues of toxins and bioregulators fall into two classes: agonists that bind to the target receptor and cause a similar response to that of the original toxin or bioregulator; and antagonists that bind to the target receptor and may block the action of the original toxin or bioregulator.

Biological warfare agent. Living organisms that are intended to cause death or disease in humans, animals or plants. They must multiply in their target in order to exert their toxic effects.

Chemical warfare agent. Chemical substances which might be employed in warfare for their direct toxic effect on humans, animals or plants.

UNITS OF MASS

One **kilogram (kg)** equals 1000 grams.

There are one thousand **milligrams (mg)** in one gram.

There are one million **micrograms (ug)** in one gram.

There are one billion **nanograms (ng)** in one gram.

There are one thousand billion **picograms (pg)** in one gram.

1.0 INTRODUCTION

1.1 Emerging Biological Threats

There is concern that rapid progress in biology, chemistry and the commercialization of biotechnology will have applications to the development of biological and toxin weapons. This paper examines the tremendous increase in our knowledge of recently identified peptides (toxins and bioregulators) that control biological activity. These advances have increased concern about the scope for misuse of toxins and bioregulators as weapons.

The Biological and Toxin Weapons Convention (BTWC), which entered into force in 1975, prohibits the development, production and stockpiling of biological or toxin weapons. The Convention does not address matters related to research. Perfectly legitimate research has continued and the advances have been especially significant, some of which could be considered relevant to the BTWC. Prior to 1975, the production of quantities of peptides that might be considered militarily significant was not possible. Scientific and commercial developments have now made it possible to produce such quantities of peptides. Since 1986, the commercialization of some of the production technologies has expanded to the point that many companies offer for sale quantities of peptides that could have military importance. The discovery of new peptides and the possibility of their large-scale production have increased the threats that the Biological and Toxin Weapons Convention has sought to contain.

1.2 Technical Progress on Peptide Toxins and Bioregulators

There has been rapid scientific progress in the following areas: identification, synthesis, modification and large-scale production of biologically active peptides.

In the past ten years, techniques for the identification of peptides, earlier considered as exceptional, have now become routine. Central to these techniques is high pressure liquid chromatography (HPLC). Without this technique it would not have been possible to isolate and identify bioregulators that exist in nanogram or picogram quantities in tissues.

The chemical synthesis of peptides has become automated. Solid-phase peptide synthesis based on the Merrifield technique is now available on a variety of commercial instruments. More recently, commercial instruments for the large-scale production of peptides have become available. Large-scale production of biologically active peptides (toxins and bioregulators) can be done by solid-phase peptide synthesis, enzymatic synthesis, or by recombinant DNA techniques. While each of these techniques has advantages and disadvantages, the choice of technique will, of course, depend on the particular peptide to be mass produced.

The most important techniques for the large-scale synthesis of small peptides are based on solid-phase synthesis. Sometimes, enzymatic synthesis is the technique of choice for the large-scale production of very small peptides, such as the artificial sweetener aspartame. This dipeptide is better known as NutraSweet, which is a registered trademark of G. D. Searle and Company Limited. In 1988, four million kilograms (four thousand metric tons) of aspartame were produced primarily by this technique. It is interesting to note that this is accomplished at the selling price of approximately one hundred dollars per kilogram. Clearly, large-scale production of peptides has become possible. However, the particular method of production may vary depending on the peptide in question.

Recombinant DNA techniques have traditionally found greater use in the large-scale synthesis of polypeptides and proteins. In the last few years dramatic changes have occurred in that new techniques have allowed peptides to be produced in new hosts in a way that was previously not possible. For example, genes coding for bioregulators have been transferred to farm animals which then produce those bioregulators in their milk. Similar experiments are also in progress for plants. Prior to this work, recombinant DNA techniques primarily used bacteria, yeast and other unicellular organisms to produce the polypeptides. These topics are discussed further in section 2.

1.3 Toxins and Biological Weapons Control

"Biological" refers to a wide variety of agents. Biological agents must infect and replicate within the target to exert their deleterious effects. (Chemical agents, on the other hand, can be lethal in a variety of ways, including when they cause massive tissue damage.) Toxins share characteristics of both chemical and biological agents. Toxins are also inanimate molecules, but they generally have a higher specificity or better fit for their cellular targets than known chemical agents. Because of their higher specificity, toxins are more potent and thus only very small amounts are necessary to interfere with physiological, that is, living processes. Toxins bind or attach to specific cellular molecules and selectively disrupt certain bodily functions. Bioregulators are small peptides that often control the release of hormones and could be considered master switches of life. Disruption or overloading of these bioregulators could lead to selective inhibition of physiological processes.

The reason why toxins are included with biological weapons in the Biological and Toxin Weapons Convention can be found in the Convention's rather circuitous historical record. Particularly significant was the fact that a major review by the National Security Council of the United States grouped toxins with biological agents in 1970 because, at that time, production processes were similar. The United States government had renounced

biological weapons in a unilateral move in 1969, and it has been suggested that this was implicitly considered to include toxins made from bacterial agents. At that time, most toxins were obtained by extraction from biological materials. However, laboratory synthesis of toxins in small amounts was emerging as an important scientific technique. Some people then questioned whether chemically synthesized toxins were also included in the USA renunciation. The subsequent review specifically examined the USA policy on toxins, and the scope of the unilateral renunciation was soon extended to include chemical synthesis of toxins for warfare purposes. This was announced by President Nixon on February 14, 1970. Clearly, this had an impact on the USA position in the multilateral consideration of the issue.

The multilateral Biological and Toxin Weapons Convention was concluded in 1972 and entered into force in 1975, and included within its scope toxins and biological agents as the shortened title above indicates. However, toxins were not defined in the text of the Convention. This was clarified to some extent in the final declaration of the Second Review Conference to the Convention which stated that, "toxins (both proteinaceous and non-proteinaceous) of a microbial, animal or vegetable nature and their synthetically produced analogues are covered." (<u>Final Declaration of the Second Review Conference of the Parties to the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and Their Destruction</u>, Disarmament (Volume 9, Number 3) 1986, page 141).

1.4 Novel Toxins and Bioregulators

Toxins are highly effective and specific poisonous chemical substances isolated from living organisms. Bioregulators are naturally-occurring chemical substances, usually peptides, involved in the regulation of metabolic, physiological and possibly neural activities. Such bioregulators have also been referred to as neuropeptides or neuroregulators.

A rapidly expanding area of research involves identifying and synthesizing new bioregulators and toxins. Synthetic derivatives or slightly modified forms of these compounds can have drastically altered and toxic effects, and this could be important in the development of novel toxic agents. Novel toxins have been discovered which are very similar in structure and function to peptide bioregulators.

A question sometimes asked is whether a modified form of a toxin would still be covered by the Biological and Toxin Weapons Convention. In principle, it may be possible to distinguish between synthetic toxins and chemicals, but it has been suggested by some that the distinction may blur when a biologically derived toxin is synthesized in vitro and then modified. If the modified toxin were similar enough to the original molecule when examined by analytical techniques, it would likely still be considered a toxin and thus fall within the scope of the Biological and Toxin Weapons Convention. There are some who suggest that difficulties in

categorization might conceivably arise if the modified toxin were changed to such an extent that it could not be shown to be modelled on a known toxin. However, such a situation, while possible in theory, is difficult to foresee in practice, given the multiplicity of known biologically active peptides.

There has been great progress in science and technology since the Biological and Toxin Weapons Convention entered into force. There has also been debate about the implications of these changes for the Convention. The United States' review leading to its renunciation of toxins concluded that toxins of that time could not surpass or replace the lethal chemical warfare agents then available. Although this conclusion may have been valid in the scientific and technical climate of 1970, thus reducing concerns about verification of a convention encompassing toxins, this may require reevaluation in the reality of the 1990s. Of course, at issue will not only be "what" some might consider needs to be done in terms of verification, but also "how" to do it.

1.5 Terminology: Distinctions and Ambiguity

The terminology "chemical and biological weapons", often mentioned together and abbreviated CBW, is misleading. It combines chemical, biological, toxin, and possibly riot control agents and herbicides. These agents have grossly different effects. Toxin agents are considered by some to blur the distinction between

chemical and biological agents. Toxins and related compounds such as bioregulators have increasingly come to be seen as posing a central difficulty in the distinction between chemical and biological warfare agents. Nevertheless, we can distinguish between biological and toxin agents. Biological agents are living microorganisms that cause infectious diseases. Toxins are poisonous chemical substances originally obtained by isolation from living organisms. Furthermore, some toxins are synthetic chemicals modelled on natural compounds.

The 1970 unilateral renunciation of biological and toxin weapons by the United States was a two-step process. The first step involved the renunciation of biological weapons. Afterwards, questions were raised about the status of toxin weapons. Toxins were generally considered to be 'chemical', rather than 'biological', agents since toxins are not capable of self-reproduction. Toxins were not even mentioned in the original renunciation of biological weapons.

Why were toxins added to the earlier renunciation of biological agents by the United States? The most persuasive reason is the similarity of large-scale production of toxins to biological agents at that time. For example, given the technology available in 1970 to make anthrax toxin, large scale production of bacterium <u>Bacillus</u> <u>anthracis</u> was a necessary first step. Since this bacterium is a potential biological warfare agent, the production of a

biological agent was necessary to produce the toxin. Other types of toxins, such as ricin and saxitoxin, could be extracted from biological materials. Most toxins suffered from the drawback that, to obtain significant amounts (from gram up to kilogram quantities), thousands of kilograms of material had to be processed. Have advances in science and technology changed this to any significant extent?

In 1970, a group of consultant CBW experts to the World Health Organization made the following observations (paraphrased from the Report of a WHO Group of Consultants, 1970. Health Aspects of Chemical and Biological Weapons, World Health Organization, Geneva, p. 26):

(1) it is unlikely that any substance appreciably more toxic than V-agents had been developed into a practical chemical weapon;

(2) there exist a large number of animal, plant and bacterial toxins, notably saxitoxin, tetrodotoxin, ricin, abrin and the bacterial toxins from Clostridium botulinum and Cl. tetani;

(3) most of these are proteins of high molecular weight that are expensive to extract and difficult to disseminate while retaining their toxicity;

(4) special circumstances could lead to their use as chemical warfare agents but it is unlikely that they would be stockpiled in preference to V-agents; and,

(5) when their toxicology is better understood, it may be possible that their toxic principles may be incorporated into more tractable substances, but this seems unlikely to happen in the near future.

There are several important conclusions to draw from these observations. First, production of protein toxins was technically possible, but it was a difficult and expensive process, particularly if one wanted to produce relatively large quantities for military purposes. Two important toxins listed above, saxitoxin and tetrodotoxin, are low molecular weight non-protein toxins from marine organisms. The group of CBW consultants to the WHO did not mention in their report that the possibility existed for chemical synthesis of these toxins, although the techniques were available in the late 1960s. Furthermore, the chemical synthesis of peptides was not mentioned. Incapacitating agents, although discussed, appear to have been discounted. As a result, some of their observations might now be open to further consideration.

There has been a tremendous number of highly potent peptides identified that control biological processes. Many of them are small peptides that are relatively easy to synthesize and manipulate chemically, and it is now feasible to produce sizeable

quantities of them. These facts must be taken into account in any consideration of the potential use of toxins and bioregulators as warfare agents, especially if physical or mental incapacitation, not only lethality, were considered to be among the range of sought-after effects.

2.0 TECHNOLOGICAL CHANGES AND PEPTIDE TOXINS AND BIOREGULATORS

This section reviews the important changes in technology used in peptide chemistry over the period 1975-1990. Included are the isolation, identification, synthesis, and large-scale production of biologically active peptides. Emphasis will be placed on methods of production.

There are two major methods that could be used to produce militarily significant quantities of peptides. First, recombinant DNA-modified microorganisms can be used to produce peptides biologically. This method is newer and holds great promise. The second method is chemical synthesis. While chemical synthesis of peptides has been possible for 25 years, the last ten years have seen major technical improvements in the accuracy of synthesis. Dramatic advances in peptide chemistry in the past ten years have also increased the capability of producing kilogram quantities of peptides. Section 2.4 gives an overview of the increases in production capabilities.

A third method of production exists, although it is more restricted in its application. Enzymatic synthesis makes use of an enzyme catalyst to form a peptide chain. For example, the dipeptide aspartame is mass produced with this technology. A limiting factor is that only very small peptides can be produced with this technique.

2.1 Peptides

The majority of novel toxins and bioregulators are peptides. In spite of their different functions, all peptides are linear polymers of different amino acids. By analogy, peptides are like beads on a string. There are over twenty possible choices for each position. Peptides consist of less than 50 amino acid residues. They differ from many other polymers in having a strictly defined length and sequence of amino acid monomers. These are acquired from their unique mode of biosynthesis. They differ from each other in that each peptide has a unique amino acid sequence. A new peptide is made every time a single amino acid is changed. These changes can dramatically alter the biological activity of the peptide.

Peptides are generally of biological origin, although it is possible to synthesize them chemically so that they have biological activity. Synthesis can confirm the structures of naturally occurring substances, and make them available in greater quantities for further investigations. It can also identify structures to allow preparation of artificial vaccines. Another result of synthesizing peptides is that it allows drugs to be made more potent by replacement of certain amino acids with others.

2.2 Genetic Engineering and Peptides

Genetic engineering has generally been equated with recombinant DNA technology. However, genetic engineering also uses many other technologies, such as DNA sequencing and synthesis, protein sequencing and peptide synthesis, monoclonal-antibody generation, microbial genetics, and computer analysis. For the purpose of this report, genetic engineering is considered to be the procedures performed on genes. These include: the isolation, expression and modification of DNA that encodes specific genes.

One of the most powerful techniques for the construction of peptides (and proteins) has been the cloning of genes. This is followed by the expression of the corresponding naturally occurring proteins in suitable host systems. Many peptides are derived from larger proteins and, therefore, cloning of the protein may be an option for production. To obtain modified protein structures, the techniques of site-directed mutagenesis have been used to modify the gene structures. Alternatively, with the considerable progress that has been made in DNA synthesis, both natural and modified proteins can be prepared through the expression of corresponding synthetic genes.

From 1975 to 1985, most work concentrated on using bacteria to express polypeptides. A number of problems exist with recombinant DNA techniques. Low yield, lack of secretion out of the host organism, and difficulty in purification are among the more

serious problems. This is partly due to incompatibility between the mammalian polypeptides and the bacterial hosts. Research has subsequently led to two advances that might overcome the difficulty of using bacteria as the host for production. Both advances depend on the transfer of genes which code for the production of the desired peptide in domestic animals and plants. Companies in North America and Europe have developed large-scale production capabilities using transgenic hosts. Although this is not a proven capability, it may hold important applications for the future.

The amino acid sequence of a given biologically active peptide usually differs slightly from species to species. However, a peptide derived from animals is usually active in humans. Some bioregulators do not require the presence of all amino acids of the parent molecule to maintain biological activity. For example, the adrenocorticotropin hormone (ACTH) is a single chain peptide containing 39 amino acids, of which the first 24 amino acids are responsible for its biological activity. In fact, when the last 15 amino acids are removed, it does not affect the hormone's biological activity.

Naturally occurring small peptides can be synthesized by direct chemical means or by recombinant DNA technology. Somatostatin is a 14-amino-acid hormone which regulates the release of growth hormone, glucagon, and insulin. It was among the first hormones made by genetic engineering and was the first for which

a totally synthetic gene was used. However, it appears that peptides with 30-35 amino acids or less are more economically produced by chemical synthesis.

2.3 Chemical Synthesis of Peptides

Although recombinant DNA techniques have captured most of the attention, chemical synthesis is emerging as an equal or superior way to produce large quantities of peptides. The Merrifield solid-phase procedure revolutionized peptide synthesis. When used in combination with purification techniques such as high-performance liquid chromatography (HPLC), the preparation of peptides 30 or 40 amino acids in length has become almost routine.

When one proceeds to the stepwise synthesis of longer peptides and of small proteins in the range of 50 to perhaps 150 amino acids in length, the problems that arise in the purification and characterization of materials often become formidable. Nevertheless, chemical synthesis of peptides continues to be the most important method. For all of these reasons, chemical synthesis of small proteins and peptides is likely to remain the method of choice.

Many techniques have been developed for the chemical assembly of amino acids to form peptides. They can be subdivided into solution and solid-phase methods. The former have evolved since the

beginning of the twentieth century. Although quite powerful, they also require considerable labour, time, and skill because of the difficulty in successfully handling the intermediates.

In 1959 R. B. Merrifield of The Rockefeller University invented the solid-phase approach. He recognized the limitations of the earlier chemical procedures but retained their strengths. Early reports showed that both manual and automated peptide synthesis was possible. Subsequent efforts from numerous laboratories in the United States, UK, Europe, Canada and Japan improved the technique of peptide synthesis.

The remainder of this section goes on to describe the procedure of solid-phase peptide synthesis. The formation of peptide bonds starts with the reaction of a tert-butyloxycarbonyl (BOC)-amino acid with a chloromethyl group on the surface of styrene polymer. After the removal of the BOC protecting group with trifluoroacetic acid, a new BOC-amino acid is allowed to react to lengthen the peptide chain, and the process repeated with a stepwise strategy. Excess reagents and by-products are removed solely by filtration and washing. Finally, the desired peptide is freed of protecting groups and liberated from the resin by anhydrous hydrogen fluoride. However, it is very difficult to avoid the production of contaminating by-products. Furthermore, these by-products are not easily separated from the desired product due to their similarity in structure and properties. Efforts continue to improve this method.

Peptides such as gastrin I, VIP, and CCK could not, until very recently, be synthesized by solid-phase peptide synthesis (SPPS). Nevertheless, solution peptide techniques allowed characterization of peptide segments (generally protected) that could be purified by crystallization.

Some biologically active peptides are readily available because they can be chemically synthesized. The synthetic replicas of the peptide represents the most economical and readily accessible source of peptides under 50 amino acids. Table 1 lists novel toxins and bioregulators that have been synthesized by SPPS.

Owing to Merrifield's solid-phase approach to peptide synthesis (SPPS), duplication of a given structure can be accomplished in a few days. Even though the homogeneity of the peptides thus generated has been questioned, a new technique of analysis and isolation (reverse-phase high pressure liquid chromatography, RPHPLC) allows an evaluation of the peptide's purity. Other valuable separation methods include ion-exchange chromatography, partition chromatography, countercurrent distribution, and affinity techniques. Proper amino acid composition can be verified using sensitive automated sequencers.

Recently, in the field of structure-activity relationships, fragments or substituted analogues have been found to be of equal or greater potency when compared to the parent molecule. They can also exhibit long-acting or antagonistic activities. This is in

marked contrast to measuring the lethality of traditional toxins. Extensive research to date on neurotoxins has shown that it is not possible to increase their potency.

Table 1.

Peptide Toxins and Bioregulators Synthesized By Solid-Phase Peptide Synthesis Process

- angiotensin II
- bombesin (frog)
- bradykinin
- cholecystokinin
- dynorphin (1-13)
- alpha-endorphin
- beta-endorphin (ovine)
- gamma-endorphin
- [Leu5]-enkephalin
- [Met5]-enkephalin
- gastrin I
- gastrin releasing peptide
- gonadoliberin
- neurotensin
- somatostatin
- substance P
- thyroliberin
- vasoactive intestinal peptide
- conotoxin G1
- endothelin
- sarafotoxin

2.4 Commercial Production of Peptides

The original methodology devised by B. Merrifield is unquestionably successful and has been employed in many laboratories. Problems identified at that level have led to improvements that have made large-scale synthesis commercially viable. One of the original motivations behind solid-phase synthesis was the possibility that the process could be automated. Equipment is now commercially available allowing the large-scale production of numerous peptides. However, it must be stressed that, even though automated systems perform all of the mechanical steps, the success of peptide synthesis is largely governed by the validity of the underlying chemistry. Also, the skill of the technicians is crucial.

Some of the most serious problems in peptide synthesis occur during removal of the peptide from the solid-phase resin (i.e. cleavage). Another problem results from impure starting amino acid derivatives. For example, amino acids are sometimes contaminated with 0.2-0.4% of the corresponding N-alpha-sec-butyloxycarbonylamino acids. This contamination occurs as a result of the amino acid modification process. Most of the contaminants can be removed, but the remaining 0.3% is enough to decrease the efficiency of peptide synthesis. Fortunately, with proper care, most of the starting protected amino acids can now be obtained with adequate (greater than or equal to 99.7%) chemical and optical purity. All

other reagents and solvents used in solid-phase synthesis must have adequate purity, lest some of the impurities react with resin-bound peptide products.

Another concern during the repetitive steps is racemization. This is the partial loss of optical purity of the starting L-amino acid during its incorporation into the peptide chain. Experiments show that, during stepwise synthesis with any of the usual N-alpha-amino protecting groups, racemization does not occur within detection limits. Some of the older techniques to couple amino acids led to production of up to 10% racemic impurities. In a synthesis where racemization is known to be confined to one or two sites, it is sometimes possible to modify coupling conditions to minimize the problem.

In sum, solid-phase peptide synthesis has matured into a proven technology. It is used throughout the scientific world. However, the procedure requires a highly skilled workforce. This is especially true for large-scale production and purification.

The first biologically active peptide prepared by solid-phase synthesis was the smooth muscle hypotensive agent bradykinin. In the last 20 years, thousands of naturally occurring peptides or their analogues have been made by solid-phase peptide synthesis. With current methods any sequence of up to 50 residues can be reliably assembled.

In the past five years, in particular, major advances have occurred in commercializing the large-scale production of peptides. Companies in the United States, Switzerland, Japan and Canada provide peptides in kilogram quantities. They produce peptides such as ACTH, calcitonin, and parathyroid hormone by solid-phase synthesis. For example, chemists in one Canadian company have developed a solid-phase peptide synthesis process capable of producing kilogram quantities of biologically active peptides up to 50 amino acids long with 98% purity. Production time is estimated at 1 to 3 weeks per batch.

In the technologies related to the synthesis and purification of biologically active peptides, there has been at least a thousand-fold improvement in production capabilities. For example, with the technologies available in 1980, peptide production would have been measured in milligram or possibly gram quantities per batch and would have required several weeks. As mentioned above, batch sizes of one kilogram are now routine, with batch production time varying from one to three weeks. Using continuous production techniques, a pharmaceutical laboratory could produce up to one kilogram per week. For a larger facility, a ten-fold scale up would seem possible given the current state of technology. This would lead to economies of scale that many say would decrease production cost from dollars per microgram to cents per milligram of biologically active peptides.

One of the principal attributes of the solid-phase method has been to make the synthesis of peptides accessible to the nonspecialist. With the increased sophistication of automated instrumentation and purification procedures, the technology is diffusing broadly. It is important, however, not to lose sight of the fact that each procedure has limits, and that even in the hands of highly experienced workers, some sequences will defy easy preparation.

Improvements in the chemistry of peptide synthesis continue rapidly. Important topics of research being investigated that will have an impact on commercial production include:

(1) increased efficiency of steps leading to shortened cycle times to attach an amino acid;
(2) better monitoring methods;
(3) preparation of protected peptide segments by mild reactions and the solid-phase assembly of the peptide segments;
(4) scale-up procedures;
(5) multiple peptide synthesis approaches; and
(6) precise control of methods for forming sulphur-sulphur bonds in synthetic peptides.

In conclusion, solid-phase peptide synthesis has developed as the method of choice for the synthesis of peptides from 3 to 50 amino acids in length. Solid-phase peptide synthesis is the method of choice. Kilogram quantities of peptides are now being produced in the time span of one to three weeks, more than a thousand-fold increase in what could be achieved less than a decade ago.

3.0 NOVEL TOXINS AND BIOREGULATORS

This section reviews work on new peptide toxins and bioregulators. The focus is on peptides that may be relevant to the Biological and Toxin Weapons Convention.

3.1 Conotoxins

Research on the conotoxin group of peptide toxins has increased significantly since its discovery in 1980. The conotoxins have novel structural features which have prompted scientific interest, and this work has caused major revisions to scientific knowledge about toxicities of small peptides. The neurotoxic conotoxins are a thousand-fold more toxic than previously characterized peptides in this size class.

The venomous fish-hunting cone snails of the genus Conus have developed a potent biochemical strategy to paralyse their prey. They produce several types of toxic peptides (called conotoxins) that attack several physiological sites. These distinct toxins share several common characteristics: they are relatively small peptides (13 to 29 amino acids); they are highly crosslinked by disulphide bonds; and they are highly basic.

Conotoxins are highly toxic to mammals and have lethal doses of approximately 10 micrograms per kilogram of body weight. This is due to the small size of the conotoxin peptides that allows for faster diffusion and transport through tissue than snake neurotoxins, resulting in faster toxic action.

Numerous peptide analogues of conotoxin GI have been prepared by solid-phase synthesis, and many have been tested in assays for their abilities to inhibit muscle contractions. It was found that tests of such modifications indicated decreased toxicity.

Another important finding that resulted from the research on the snail venom was the isolation of a peptide homologous to the mammalian bioregulator vasopressin.

3.2 Sarafotoxin-Endothelin

There is a growing number of peptide toxins which are similar or identical in structure to naturally occurring bioregulators or "hormone-like" compounds. This points to the important role that bioregulators will have in the identification of novel toxic compounds. For example, sarafotoxin isolated from snake venom is identical in structure to the bioregulator endothelin. Sarafotoxins are a group of 21-residue cardiotoxic peptides isolated from snake venom. They induce coronary vasoconstriction. They show high-affinity binding to rat atrial and brain membranes. Neither

their binding nor their activity is affected by blockers or activators of known receptors and ion channels. This suggests that sarafotoxins act on new targets. Their amino acid sequence shows a high degree of homology with that of endothelin.

Endothelin is a recently described 21-residue vasoconstrictor peptide found in porcine aortic endothelium. This is remarkable, since endothelin is a natural compound of the mammalian vascular system, while sarafotoxins are highly toxic components of snake venom.

Sarafotoxins S6 (SRTs a, b, and c) are a group of 21-residue cardiotoxic peptides that were isolated from the venom of the snake Atractaspis engaddensis. They are rich in cysteine (four residues per molecule) and show a high sequence homology. Two of these, a and b, are lethal and cause cardiac arrest and death in mice within minutes of intravenous administration. The median lethal dose is approximately 15 micrograms per kilogram of body weight.

3.3 Bioregulators

There are a number of human polypeptides of potential interest. The following is a partial list of these bioregulators, with their size in amino acid residues shown in brackets. They are: insulin-like growth factors [67,70]; thymopoietin [49]; gastric inhibitory polypeptide [43]; corticotropin [39]; cholecystokinin

[33]; calcitonin [32]; endorphins [31]; glucagon [29]; thymosin-alpha 1 [28]; secretin [27]; and motilin [22]. These peptides have been made by chemical synthesis.

Naturally occurring small peptides being studied, with amino acid residues in brackets, are: dynorphin [17]; somatostatin [14]; bombesin [14]; melanocyte-stimulating hormone [13]; neurotensin [13]; angiotensin I [10]; bradykinin [9]; vasopressin [9]; oxytocin [9]; angiotensin II [8]; angiotensin III [7]; enkephalins [5]; and thyrotropin-releasing hormone [3].

The brain and the gastrointestinal tract contain numerous peptides which exert a variety of endocrine, central nervous system, behavioural, and peripheral actions. Such powerful biological actions indicate that these compounds are a new class of neurotransmitters. Some of these peptides have been isolated from brain extracts and have been characterized, whereas others have only been localized in the brain by radioimmunoassay and/or bioassay. They can be found in the tissues of a number of mammalian as well as nonmammalian species, and their structures usually are very similar.

The characterized peptides are easily replicated by synthesis. In some cases this has led to the design of analogues with specific biological activities, such as prolonged duration of action or antagonistic behaviour. They are presently used as tools to

provide a better understanding of the mechanisms governing the systems on which they act. These types of bioregulators are beginning to be used clinically for replacement therapy and in some tests.

The numerous peptides that have been identified in the central nervous system exert gastrointestinal and behavioural effects as well as modify endocrine function. Most of these peptides have been isolated and characterized from discrete anatomical areas on the basis of well-defined biological activities. Thyrotropin-releasing factor (TRF), luteinizing-hormone-releasing factor (LRF), and somatostatin (SS) were characterized as being secreted from the hypothalamus on the basis of their ability to influence certain hormones. Immunofluorescent assay, immunoreactivity, and/or biological activity revealed TRF and SS to be distributed throughout the brain and the gastrointestinal tract. These biologically active peptides have emerged as a class of new extracellular messenger substances which may play an important role in the transmission of information and in the regulation of physiological mechanisms.

With the development of Merrifield's solid-phase approach to peptide synthesis, duplication of a given structure can usually be accomplished in a few days. New techniques of analysis and isolation, such as reverse-phase high pressure liquid chromatography (RPHPLC), allow precise evaluation of a peptide's

purity. As was mentioned earlier, recent work in the field of structure-activity relationships has demonstrated that fragments or substituted analogues are of equal or greater potency, except in the case of neurotoxins, when compared with the parent molecule. In addition, they may exhibit long-acting or antagonistic activities.

The following sections describe bioregulators that have been the subject of extensive research. Much of the material can be found in such common sources as The Merck Index (Third Edition) and The Kirk-Othmer Encyclopedia of Chemical Technology (Third Edition).

3.4 Substance P

Substance P was originally detected in the acid-alcohol extracts of equine brain and intestine. It was subsequently isolated from bovine hypothalami. It is also present in the spinal cord, in the gastrointestinal tract, and in mammalian lung. Substance P was first isolated for its hypotensive action and stimulation of rabbit jejunum contraction. Later it was found to produce salivation in rats. Substance P also stimulates glucagon secretion and produces hyperglycemia in the rat. It stimulates smooth muscle contraction in the guinea pig vas deferens and ileum, and elevates GH and PRL secretion.

The sequence of isolated endogenous substance P was confirmed through synthesis of the peptide by solid-phase peptide synthesis using a benzhydrylamine resin which, upon HF cleavage, yields the C-terminus amide directly. The biological properties of both the synthetic and the natural substances are qualitatively and quantitatively identical. The smallest sequence possessing most of the Substance P spectrum of activity and high potency is the hexapeptide C-terminus.

3.5 Thyroliberin (TRF)

Thyroliberin (TRF) originally was purified from alcohol-acid extracts of ovine and porcine hypothalamic extracts. Its greatest concentration is found in the mammalian central nervous system. It is also present in the blood, urine, cerebrospinal fluid, and endocrine pancreas. A macromolecule similar to TRF (as determined by physical and immunochemical properties), and that generates TRF upon trypsin and carboxypeptidases digestion, has also been reported in frog-brain extracts.

TRF's ability to stimulate TSH secretion in the rat and mouse justified its purification from hypothalamic extracts. It was subsequently found to release prolactin (PRL) and GH under specific conditions. TRF has been reported to alleviate depressive symptoms. It also reportedly reverses the duration of anesthesia and hypothermia induced by a number of substances. It may also increase

spontaneous motor activity. As a neurotransmitter candidate, it modifies the rate of discharge of neurons and the secretion of monoamine analgesics, such as hypnotics, sedatives, and anticonvulsants.

This potent, but structurally simple, peptide was first isolated from hundreds of kilograms of brain extracts to yield only milligram quantities of peptide. Upon hydrolysis of the highly purified ovine and porcine extracts, only three amino acids were found in equimolar amounts. As an approach to TRF's characterization, all possible permutations of these three amino acids were synthesized and yielded six peptides, none of which was active. Upon cyclization of the N-terminus glutamic acid and amidation of the C-terminus proline in the sequence of Glu-His-Pro, a fully blocked tripeptide was obtained that had all of the characteristics of the natural hormone, including its biological activity. To date, innumerable syntheses have been reported which have included classical and solid-phase approaches. Several hundred analogues of TRF have been synthesized. Some of the analogues are more potent than others, while some are selective in action on the central nervous system.

3.6 Gonadoliberin (LRF)

Gonadoliberin (LRF) was isolated from porcine and ovine hypothalamic extracts. Its highest concentration is found in the hypothalamus. However, it is also reportedly present in extrahypothalamic central nervous regions, blood, urine, and placenta. LRF acutely stimulates LH and FSH secretion. Single injections induce ovulation and increase steroid secretion. Additionally, there is evidence that it can act on the central nervous system to modulate sexual behaviour. Paradoxically, its long-term administration is associated with antigonadal effects, which include termination of pregnancy, decreased gonadal weights, and lowered steroid secretion. This is possibly due to desensitization at pituitary and gonadal levels and through alterations in steroidogenesis.

The sequence of isolated endogenous LRF was confirmed by synthesizing the decapeptide using solid-phase techniques. Once purified, the synthetic product showed the same physicochemical and biological properties as the natural porcine LRF.

Potent and long-acting analogues of LRF have been designed. These peptides generally have a D-amino acid at the 6-position. The LH-releasing potency of the most potent agonists (i.e. analogues which have similar biological effects) are about one hundred and fifty times that of LRF. Potent antagonists also have been

designed. Analogues can inhibit ovulation in the rat at a single dose of 20 micrograms. They can also terminate pregnancy and inhibit spermatogenesis in the rat.

3.7 Somatostatin (SS)

Somatostatin (SS) was isolated from ovine hypothalamic extracts because of its characteristic inhibition of the spontaneous release of GH by cultured pituitary cells. Additionally, SS inhibits GH secretion that is mediated by most known secretagogues. It also inhibits a large number of other secretions, such as TSH, PRL, insulin, glucagon, acetylcholine, gastrin, gastric acid and other digestive enzymes. SS is distributed throughout the brain and the gastrointestinal tract. It also is found in mammalian plasma, in the rat retina, and in the human adrenal medulla. It appears to exist in longer forms in several biological tissues. Some of them may represent precursors of the tetradecapeptide.

One of the longer forms, which was isolated from porcine intestine, has been characterized as a 28 amino acid peptide. Its similarity to the larger forms of brain somatostatin and its possible physiological role is unclear. The 14 amino acid peptide has been isolated and characterized from the pigeon pancreas, the anglerfish pancreatic islet, and the rat extrahypothalamic brain. SS from the latter sources is identical to the mammalian hypothalamic tetradecapeptide SS.

Somatostatin exerts some neurotropic actions, e.g. as a tranquillizer and as a spontaneous motor activity depressor. It also lengthens barbiturate anesthesia time and induces sedation and hypothermia.

As a final proof of its structure, somatostatin was synthesized using solid-phase techniques. Once purified, the synthetic product had all the physicochemical and biological properties of the natural product.

Substitution with selected amino acids increased the potency of somatostatin and all other active analogues by a factor of eight. Most other substitutions, however, are deleterious to biological activity. Analogues with the Asn5 residue deleted had selective effects on the inhibition of insulin and were one thousand seven hundred and fifty times more potent in inhibiting insulin than in inhibiting glucagon secretion by the pancreas. Similarly, substitution of Cys14 by D-Cys14 gave an analogue that is more selective for the inhibition of glucagon secretion than that of insulin. Cyclic octapeptide analogues of somatostatin retain high potency.

3.8 Neurotensin (NT)

Neurotensin (NT) was isolated and characterized from acid-acetone extracts of bovine hypothalamus on the basis of its hypotensive activity. Immunoreactive neurotensin is present in

mammalian gut and distributed throughout the central nervous system. Its highest concentration is in the hypothalamus.

The many pharmacological actions of neurotensin include induction of hypotension, increased vascular permeability, hyperglycemia, increased intestinal motility, and inhibition of gastric acid secretion. Its effects on insulin, glucagon, and SS secretion appear to depend on glucose concentration. Its effects on prolactin, growth hormone, and gonadotropin secretion seem to depend on whether it is administered intravenously or intracerebrally.

The sequence of isolated endogenous neurotensin was confirmed by synthesizing the tridecapeptide using solid-phase techniques. The data obtained from the synthesis demonstrates that the purified synthetic product is chemically and biologically indistinguishable from the isolated hypothalamic substance.

The smallest sequence possessing most of the neurotensin spectrum of activities as well as its high potency is the hexapeptide C-terminus of NT. A series of analogues has been synthesized. in which each amino acid is substituted by its corresponding D-amino acid. This series yielded analogues with biological activities ranging from 0 to 1000% of the parent compound.

3.9 Bombesin (BN)

Bombesin (BN) was first isolated and characterized from methanol extracts of frog skin. Subsequently, bombesinlike activity has been found by immunochemistry bioassay and radioimmunoassay and throughout mammalian intestine, lung, brain, plasma and gut. A peptide (gastrin-releasing peptide, GRP) with a striking homology in the C-terminus region of bombesin, also has been isolated and characterized from porcine gastric tissue. It remains to be shown that brain bombesin, which appears to have a higher molecular weight than frog skin bombesin, is GRP.

Bombesin is related to a family of biologically active peptides which differ in the interchange of phenylalanine for leucine in the penultimate C-terminus position and in the variable number of amino acids extended towards the N-terminus. Isolation of bombesin was accomplished by following its stimulant action on secretion from the denervated fundic pouch of the dog. It releases gastrin, gastric acid and cholecystokinin. It also increases pancreatic secretion, produces contraction of the gall bladder, and increases blood pressure. It also produces hypothermia in rats exposed to a cold atmosphere. Its intravenous administration stimulates PRL and GH secretion, but reportedly inhibits basal PRL secretion after central administration.

The relative potency of bombesinlike peptides was reported using different biological systems. Short-chain analogues are significantly more potent than bombesin.

3.10 Endorphins and Enkephalins

A group of peptides possessing part of the structure of beta-lipotropin (beta-LPH, which is a 91-amino acid protein) have morphine-like properties.

The active endorphins and enkephalins are:

(a) the C-fragment (beta-endorphin, residues 61-91 of beta-LPH) isolated from the pituitary and also present in the brain;
(b) the C-fragment [beta-LPH 61-87] present in the pituitary;
(c) gamma-endorphin [beta-LPH 61-77];
(d) alpha-endorphin [beta-LPH 61-76] extracted from hypothalamic and pituitary tissues;
(e) Met-enkephalin [beta-LPH 61-65]; and
(f) Leu-enkephalin, which is localized in the intermediate and anterior pituitary lobes only.

These peptides specifically displace bound naloxone from brain opiate receptors. Beta-endorphin is more potent than the short peptides. Additionally, the peptides elicit a number of morphine-

like activities following cerebroventricular injection, e.g. analgesia and catatonia. Behavioural effects are also exerted. In vitro, they decrease the amplitude of muscle contractions induced electrically in the guinea pig ileum and in the mouse vas deferens. All of these effects are reversed by the opiate antagonist, naloxone.

A number of peptides possessing naloxone-reversible opioid activity, but distinct from beta-endorphin and the enkephalins, have been reported in pituitary and hypothalamic extracts and in human blood.

Hundreds of enkephalin analogues have been synthesized in an effort to find a nonaddictive opiate. Among the structures showing higher potency are those having a D-alanine at the 2-position, an N-methylated phenylalanine at the 4-position, and methioninol sulfoxide at the C-terminus. The pentapeptide exhibited definitive analgesic activity (even after oral administration). It was about 30,000 and 1,000 times more potent when injected than Met-enkephalin and morphine, respectively, and twenty-three times as active as beta-endorphin. Whereas most analogues were found to be opiate agonists, evidence for an antagonistic nature of N-Allyl-Leu5 -enkephalin was given.

Synthetic alpha- and gamma-endorphins are the same as the respective natural substances in terms of the following tests:

(1) amino acid composition;
(2) HPLC pattern;
(3) mobility (Rf) values on thin layer chromatography in different solvent systems;
(4) mass spectra after derivatization; and
(5) biological activity.

Many analogues of beta-endorphin have been synthesized. One of the most interesting approaches to analogue design is that of trying to make cyclic analogues of native linear substances in order to stabilize a tertiary structure and to better understand the topographical requirements of the receptor for recognition and transduction. From the synthetic chemist's point of view, cyclization by introduction of two half-cystine residues, which can be coupled under mild oxidizing conditions to form a disulphide bridge, is the easiest approach to such structures. This was applied in the design of several LRF and NT analogues that were found to have low, but significant, biological activity. Two out of three of the cyclic analogues are equipotent or even more potent than beta-endorphin in biological assays.

Beta-endorphin has been produced by bacterial synthesis via a genetic-engineering technique. As well, it has been synthesized by solid-phase synthesis.

3.11 Dynorphin

The partial primary structure of dynorphin, a novel porcine pituitary endorphin having the N-terminus sequence of Leu-enkephalin, has been disclosed (13 out of 17 amino acids could be sequenced). The synthetic replicate of the 13-peptide is seven hundred times more potent than Leu-enkephalin and fifty times more potent than beta-endorphin in biological assays. The high potency and the considerable immunoreactivity that this peptide displays in assays with antisera have been used for the immunohistochemical localization of Leu-enkephalin.

3.12 Oxytocin and Vasopressins

Oxytocin and the vasopressins, 8-arginine and 8-lysine vasopressin, are the major posterior-pituitary hormones found among the higher mammals. These nonapeptides exert a variety of physiological effects, such as milk ejection and uterine contraction by oxytocin, and pressor action and an antidiuretic effect for vasopressins. Synthesized in the hypothalamus, these hormones are attached to their carrier proteins, the neurophysins, and travel to, and are stored within, the posterior-pituitary lobe.

Based partly on the molecular modelling of the peptides, many highly potent agonists and inhibitors have been synthesized by both solution and solid-phase methods of peptide synthesis. Although subject to rapid enzymatic degradation, these peptides have found substantial uses in medicine to induce labour, to treat diabetes insipidus, and to promote milk secretion. It has been suggested that vasopressins are involved in memory retention and consolidation.

3.13 Other Peptides

A number of peptides have been reported in various tissues by radioimmunoassay and bioassay. Gastrin [17 amino acids] and vasoactive intestinal polypeptide [VIP; 28 amino acids] have been reported to be present in human and canine brain, respectively, and throughout the gastrointestinal tract. Cholecystokinin [CCK; 33 amino acids] has been found in the brain and intestine. Renin and angiotensin-forming enzyme have also been found in brain areas, including the hypothalamus and the cerebellar cortex, respectively. Delta sleep-inducing peptide [DSIP; 9 amino acids] shows enhancement and induction of delta (slow-wave) brain activity similar to sleep when administered to mammals.

Chemical and pharmacological tests have revealed the presence of angiotensin and renin in rat and dog brain. Although bradykinin was isolated from blood on the basis of its ability to slow contraction of the guinea-pig ileum, bradykininlike immunoreactive

structures have been localized in rat brain by immunofluorescence assay. Pentagastrin is among the most used gastrin analogues and has all of the biological properties of gastrin I. Similarly, the tyrosine sulfated and nonsulfated octapeptide C-terminus of CCK has most of the CCK biological properties, including high potency. Potent angiotensin analogues of bioregulators such as the antagonist [Sar1,(alpha-Me)Ala8]-AngII have been developed. Although the use of peptide bioregulators or selected analogues offers great potential, to date few such analogues have been used in humans, usually because of their short duration of action. However, research and development is in a stage of major growth. TRF, LRF, and SS are administered together in a standardized test to study pituitary function. However, their short duration of action and lack of complete specificity have limited their therapeutic usefulness. TRF has been reported by some investigators to relieve depression; however, most studies of the use of TRF in depression have produced negative results. It is hoped that somatostatin can be used as a substitute for insulin therapy in diabetes and as a means of lowering glucagon secretion. However, its short half-life and its widespread effects on numerous functions have prevented its clinical usefulness. The main interest of somatostatin is in its role in turning off the secretion of catecholamines and in lowering blood pressure. The only analogues used by several groups of clinical investigators as a means of improving pathological conditions are long-acting LRF agonists.

4.0 POTENTIAL DANGERS DUE TO CHANGES IN TECHNOLOGY

This section of the paper reviews potential dangers due to changes in technology related to novel toxins and bioregulators. These dangers include potential changes in military utility, proliferation, and new incapacitating agents. Extrapolation of current technological trends will also be discussed.

4.1 Changes in Potential Military Utility

As was detailed in the previous sections, there have been three interrelated scientific and technical developments which have a significant impact on any discussion of novel toxic compounds. These developments include:

(1) scientific advances, resulting in the development of several methods for the large-scale production of biologically active peptides;

(2) dramatic technical advances in the world-wide biotechnology industry, resulting in commercially validated processes involved in the production, purification and delivery of peptides; and,

(3) the beginnings of a scientific revolution in the understanding of the role of peptide bioregulators in controlling biological processes.

Obviously, the first two developments have built on each other and led to increased interest in the area as a whole. A major impact of these developments is that it is now possible to produce quantities of biologically active peptides that could be used for military purposes. This was not possible before 1980. Especially since 1986, many companies have established large-scale production facilities and advertise kilogram quantities of bioregulators. These include companies in Switzerland, Denmark, Canada and the United States, to mention a few.

Over the past five years, the significance of the third development stated above has become more evident. Given the pace of research, concern has emerged about the potential for abuse of these novel peptides. Bioregulators are thought by some to be especially important, since it is said they could be used as incapacitating weapons.

Another important factor is the possibility of making derivatives of the biologically active peptides. Chemical synthesis techniques allow selective modification of the peptides. Published research on bioregulators has shown that it is possible to make modifications which significantly increase the activity of the peptide. This would decrease the dose necessary for the biological effect. As well, the duration of action of the bioregulator can be modified by changing its rate of degradation. Because bioregulators have many different sites of action, this gives rise to the possibility of selectively affecting mental processes and many

aspects of health, such as control of mood, consciousness, temperature control, sleep or emotions. Even a small imbalance in these natural substances could have serious consequences, inducing fear, fatigue, depression or even causing death.

There is no evidence to date that toxins isolated from bacteria, venoms and plants, and having a lethal effect on living organisms, can have their toxicity increased by replacement of amino acid residues. There appears to have been an evolutionary selection for lethal toxins in particular organisms, giving the latter an advantage for survival. Therefore, for lethal toxins interacting at a specific cellular target, a limit has been reached which cannot be overcome by modifying the toxin. With bioregulators, this is not the case since these compounds are involved in modulating cellular activities. They do not have a single endpoint of function as neurotoxins do. The significance of this is that, while it is unlikely that research may lead to more toxic lethal agents, it may be possible to make more effective incapacitating agents. In this regard, examining the lethal dose of an incapacitating compound may not give an adequate representation of its potential as a toxic agent. A more significant indicator would be the effective dose at which it would act as an incapacitant.

Science and technology are closely interrelated, and it is certain that these trends are being monitored by those laboratories charged with research on novel toxic compounds. But there are

separate concerns about technology in general and, in particular, about technological breakouts that could have a major political or military impact. One such scenario involves a technological breakthrough in the development of a capability based on a toxic agent with properties which cannot be achieved with currently known or stockpiled chemical warfare agents.

In this context, concerns about toxins can be described in the following way. A country not constrained by international convention could develop a toxin with a toxicity several hundred times greater than current chemical weapons but having the necessary stability for field dissemination, and no significant delay in onset of effect. Another possibility might be that a toxin could be developed which is as toxic as presently stockpiled nerve agents, but is not stopped by filters in currently used protective equipment. Some characteristics of new agents that would offer significant advantages over existing toxic agents include:

(1) novel sites of toxic action;
(2) rapid and specific effects;
(3) penetration of protective filters and equipment; and,
(4) militarily effective physical incapacitation.

Despite the important scientific and technical advances in the synthesis of peptides, some cautions should be noted. First, it is now technologically possible to produce relatively large quantities

of biologically active peptides. However, it still requires a major research and development program. It goes without saying that production is not the sole requisite for a military capability.

There are stringent requirements necessary for field dissemination. Information in the open literature about stability of peptides in non-laboratory conditions is not readily available. It is possible that these novel compounds are sensitive to environmental effects such as temperature, wind and humidity. Protein toxins, such as botulinum toxin, have been shown to be very sensitive to such effects. For example, botulinum toxin inactivates readily and loses its toxicity in field dissemination. While this may be a problem for large protein molecules, the peptides discussed above may not sensitive in the same way. Small peptides, especially those which have been modified to have a circular structure, have higher stability and are more resistant to inactivation than the larger proteins.

4.2 Proliferation

The possibility exists that countries or small groups could conduct secret research and development of weapons based on toxins or bioregulators. However, the use of new sophisticated technology requires specialized and expensive equipment, and qualified personnel. These factors would provide an effective barrier to research by anyone with limited resources. Of course, it must be

kept in mind that this type of research and development is significantly less expensive and requires a less skilled workforce than, say, acquiring a nuclear warfare capability.

It is far more likely that would-be proliferators would try to purchase key equipment. Large-scale peptide synthesizers, chemical reagents for synthesis, and chromatographic equipment for purification can all be purchased from a variety of commercial sources. It is also possible to purchase kilogram quantities of biologically active peptides which could be diverted to purposes prohibited by the Biological and Toxin Weapons Convention.

4.3 Novel Toxins and Bioregulators as Incapacitating Agents: The Evolution of a Novel Category of Agents

The idea of incapacitating weapons is an old one. However, with the increased knowledge and use of bioregulators, the whole concept of incapacitating weapons will have to be reexamined.

For the purpose of analysis, one must distinguish between lethal and incapacitating weapons. The United States and the Soviet Union, for example, have stockpiles of lethal chemical agents. As for incapacitating weapons, publicly-available documents indicate that the US Army had strict criteria which put these types of potential weapons in a special category. They ruled out the use of lethal agents at sub-lethal doses. Substances that cause permanent

or long-lasting injury, such as blister agents and those causing eye injury, would not qualify as incapacitating weapons. The basic purpose of an incapacitating agent was to reduce military effectiveness without endangering life.

It is at this point that the importance of bioregulators or agents of biological origin take central importance. Bioregulators could give capabilities that classical chemical or toxin agents cannot give. The prime effect would be physical incapacitation that would not lead to death. Lethality is a reliably measured endpoint; however, there are a myriad of other biological effects, some of which may be militarily significant.

4.4 Extrapolation of Current Technological Trends

This section extrapolates current scientific and technical trends over the short and medium term. In the short term, improved biological production of active compounds can be expected. Chemical synthesis will remain an integral part of large-scale production of peptides. The future of biologically based methods of peptide production will see an increased emphasis on the use of transgenic plants and animals.

In the long term, development of highly specific analogues of naturally occurring bioregulators or toxins can be expected. The development of militarily effective physical incapacitation that

is reversible may be possible. However, this will require substantial research and development.

The assertion has frequently been made that advances in technology can provide the means to produce large quantities of toxins that previously were not possible. The production of protein toxins in quantities that could be considered as militarily significant, such as ricin, botulinum toxin A and staphylococcal enterotoxin B was possible before 1970. Only in the synthesis of small peptide toxins, such as conotoxin and bioregulators, has a significant change occurred. The automated coupling of amino acids to form peptides has been developed. However, large-scale production of quantities in hundreds of kilograms has not been reported and would be a major technical, as well as expensive, undertaking.

As was described in a previous section, neurotoxins isolated from natural sources cannot be made more toxic. This imposes a fundamental constraint. This is in marked contrast to bioregulatory peptides. Analogues of bioregulatory peptides that are hundreds or thousands of times more potent than their parent molecules could be developed. This could make these molecules the most potent chemicals affecting living processes.

5.0 CONCLUSION:

IMPLICATIONS FOR THE BIOLOGICAL AND TOXIN WEAPONS CONVENTION

Toxins and bioregulators, it is often suggested, are in an ambiguous position with respect to chemical and biological weapons. Some people consider toxins and bioregulators to be biological agents, while others consider them to be chemical agents. Accordingly, there is a view that toxins and bioregulators should be included not only in the Biological and Toxin Weapons Convention but also in the proposed Chemical Weapons Convention, to ensure that there not be any perceived gaps in the scope of the prohibition. The advances in technology related to novel toxins and bioregulators have contributed to such concerns. Biologically active peptides could require further consideration in the years ahead.

However, if the scope of the Biological and Toxin Weapons Convention is properly interpreted to include bioregulators (such as biologically active peptides) and novel toxins, any verification of activities relating to these would not be a simple matter. Pharmaceutical plants around the world have incorporated safety provisions akin to those which were once unique to biological weapon production facilities, making it increasingly difficult to distinguish between permitted and prohibited activities. Verification of a biological or toxin weapon production capability would require a high degree of intrusion. In other words, all

suspect facilities would have to be subject to careful inspection. It could be extremely difficult to obtain the necessary access to inspect properly all the potential sites. Further, there are now many more facilities capable of producing large quantities of toxins and bioregulators than existed in the 1970s when the Convention entered into force. While the large-scale synthesis of some potent peptides may have legitimate medical or scientific uses, the possibility exists for diversion for illegal purposes.

Without on-site verification, illegal activity would be more difficult to detect. One would then have to rely on other forms of monitoring to keep abreast of what is occurring. Examination of the open scientific literature is one method that is available to monitor this area. Patterns of research can be established, interesting trends monitored and, most importantly, abrupt halts in the research on a particular topic can be revealing. Monitoring of the literature, coupled with other sources of information, can point to questionable activities. For example, countries rather abruptly devoting inordinate resources to biotechnological research on toxins and bioregulators may be suspect.

In summary, technological changes in the past few years have an important bearing on the issue and potential scope of verification of the Biological and Toxin Weapons Convention. There has been a tremendous increase in the identification of peptides, both toxins and bioregulators, that control biological processes.

This has dramatically increased the possibility of finding potential warfare agents with new sites of action that are radically different from known agents. This is especially true for bioregulators. In addition, technological advancement makes possible the production of quantities of toxins and bioregulators previously not possible. These quantities may be considered by some to be militarily significant. If so, this could further the potential interest in the development of toxins and bioregulators as weapons. All of these changes have contributed substantially to the difficulties that would need to be faced with regard to any amendment of the Biological and Toxin Weapons Convention to include provision for verification. Solutions to such problems will not easily be found nor negotiated.

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